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Molecular Cloning and Sequence Analysis of *gdhA* Gene of *Pasteurella multocida* B:2

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Abstract: *Pasteurella multocida* has been recognised as an important pathogen of animals that causes a wide spectrum of diseases, collectively termed as pasteurellosis. The bacteria possess several housekeeping genes that maintain the viability of the bacteria. One of the housekeeping genes is the *gdhA* gene that serves as a major link between carbon and nitrogen metabolism. The full length *gdhA* gene of *P. multocida* B:2 was found to be 1180bp while the functional fragment of the gene was 652bp. The gene was successfully sequenced and later cloned into *E. coli* pCR 2.1-TOPO vector.

Key words: *Pasteurella multocida* B:2; *gdhA* gene

INTRODUCTION

Pasteurella multocida is the causative agent of a wide range of diseases in both wild and domestic animals leading to significant economic losses worldwide. It has been recognised as an important pathogen that causes major problems in ruminant industry[1] . Among the most important disease is haemorrhagic septicaemia, which is caused *P. multocida* B:2 [2].

The bacteria possess several housekeeping enzyme genes that play important roles in maintaining the viability of the bacteria[3]. Based on the multilocus sequence analysis of housekeeping enzyme genes, *P. multocida* is known to possess seven common housekeeping enzyme genes that are interspersed around the chromosome[4]. The genes that codes for housekeeping enzymes and involves in nucleotide biosynthesis are *adk* (adenylate cyclase) and *deoD* (purine nucleoside phosphorylase), while *aroA* (3-phosphoshikimate 1-carboxyvinyl transferase) and *gdhA* (glutamate dehydrogenase) play a role in amino acid biosynthesis. In addition, other genes that involve in energy metabolism pathway such as pentose phosphate pathway, TCA cycle and glycolisis pathway are *g6pd* (glucose-6-phosphate 1-dehydrogenase), *mdh* (malate dehydrogenase) and *pgi* (phosphoglucose isomerase)[4].

The glutamate dehydrogenase gene *(gdhA)*, other than catalyses the reversible deamination of L-glutamate to a-ketoglutarate, also serves as the major link between carbon and nitrogen metabolisms[5]. These inter-conversions are catalysed by several enzymes, which include glutamate synthase, glutamate dehydrogenase and glutamate-dependent aminotransferases[6]. In *P. multocida,* the transcription of *gdhA* gene produces glutamate dehydrogenase enzymes that play a role in amino acid transportation[4]. From the previous study on the *gdhA* gene, manipulation of this specific gene would not disturb the viability of the bacteria since it only alters the pathway of certain amino acids that cause the organism to be glutamate dependant[3]. This report identifies and describes the sequences and cloning of the *gdhA* gene of *P. multocida* B:2.

MATERIALS AND METHODS

Bacterial strain: Two strains of *Pasteurella multocida* B:2; the PmTB and Pm3030, isolated earlier from outbreaks of haemorrhagic septicaemia of cattle, were grown either in Brain Heart Infusion (BHI) broth (Oxoid) or on blood agar containing 5% (vol/vol) sheep blood and 1.2% (wt/vol) agar. It was incubated at 37°C for 24 h and shaken at the rate of 150 rpm when grown in brain heart infusion broth.

Escherichia coli were grown either in Luria broth flasks at 37°C and shaken at 150 rpm or on Luria agar containing 1% (wt/vol) agar. Ampicillin was used at the concentration of 50 μg m L^{-1} when needed[7].

Preparation and manipulation of DNA: Chromosomal and plasmid DNA of *P. multocida* B:2 were isolated using the standard procedure. The DNA concentrations were determined by UV-spectrophotometry at 260 and 280 nm (Ultrospec 2000, GE Healthcare Bioscience, USA).

Amplification of the gene by PCR and nested PCR: The chromosomal DNA was amplified by PCR using specific

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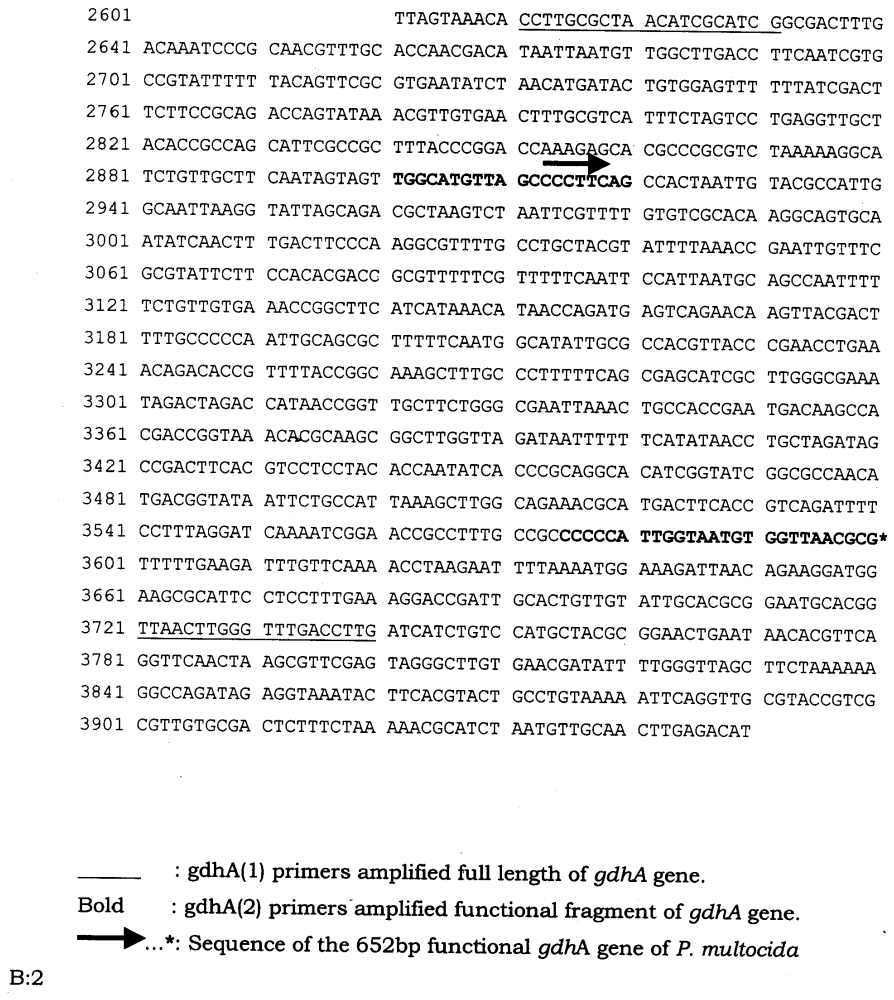

Fig. 1: Locations for both sets of primers, gdhA(1) and gdhA(2)

primers gdhA(1), 5'- CTTAGTTGAACCTGAACG-3' for sense primer and 5'- CTTGACCTTCAATCGTGC -3' for anti-sense primer. Nested-PCR was then completed using the earlier PCR product with gdhA(2) primers, 5'-CGCGTTAACCACATTACC-3' for sense primer and 5'-CCCTTCAGCCACTAATTG-3' for anti-sense primer to target amplification at the *gdh*A coding region Fig. 1[4]. PCR was carried out with materials provided by Fermentas, which contained 5nmoles of each deoxynucleoside triphosphate, 0.8 µmoles of KCI, 0.2 µmoles of Tris-HCL (pH 9.0), 0.03 µmoles of $MgCl_2$ and 1 unit of Taq DNA polymerase. The bacterial DNA and 25 pmoles of each primer were then added to the PCR master mix tube. PCR was then carried out in a final volume of 15 uL. Both PCR reaction were run for 35 cycles on a Eppendorf Personal Master Cycler under the following conditions: 1 cycle of denaturation at 95°C for 4 min, followed by 40 cycles of 95°C for 1 min, 56- 57°C for primer annealing for 1 min and extension of 72°C for 1 min. The final cycle included another additional extension step of 72°C for 10 min.

Cloning, sequencing and analysis of the amplified *gdhA* gene: Ensuing the existence of both amplified DNA fragments on a 1% agarose gel electrophoresis, both PCR products were fractioned by AGE, excised from the gel and purified using the Gel Extraction Kit (Qiagen, UK). The purified PCR fragments were then sequenced in both direction on an ABI Prism 377 automated DNA sequencer (Applied Biosystem, Foster City, USA) before the results were compared with the whole genome of *P. multocida* serotype A (PM70) from NCBI by BLAST (http://www.ddbj.nig.ac.jp/search/blast-j.html). Subsequent to confirmation, both PCR products were cloned into pCR2.1 TOPO and transformed into *E. coli* TOP10. The propagated plasmids were then prepared

using QIAprep Spin Miniprep Kit (Qiagen, USA). All positive clones were identified by restriction enzymes digestion with *Bam*HI and *Xho*I (Fermentas, Lithuania). Both nucleotide sequences were analyzed using the BioEdit v.5.0 software.

RESULTS

Characterization of the gdhA gene of *P. multocida* B:2: The first pair of primer (*gdh*A (1)) successfully amplified the full length of *gdh*A gene, which was 1180bp Fig. 2. The second pair of primer (*gdh*A[2]) amplified the functional fragment of the *gdh*A gene, which was 652bp (Fig. 3).

***gdhA* sequence:** The 652bp functional *gdh*A gene of *P. multocida* B:2 was sequenced and was found to be 98.7% homologous with the respective gene in the genome sequence of *P. multocida* A (Pm70) used in haemorrhagic septicaemia vaccine preparation (Fig. 1).

Cloning of the PCR product: Following cloning, treatment of recombinant pSZ1 that consisted of pCR2.1-TOPO vector and the *gdh*A gene with *Bam*HI and *Xho*I confirmed the existence of 1180bp gene and determined the orientation of the insert (Fig. 4).

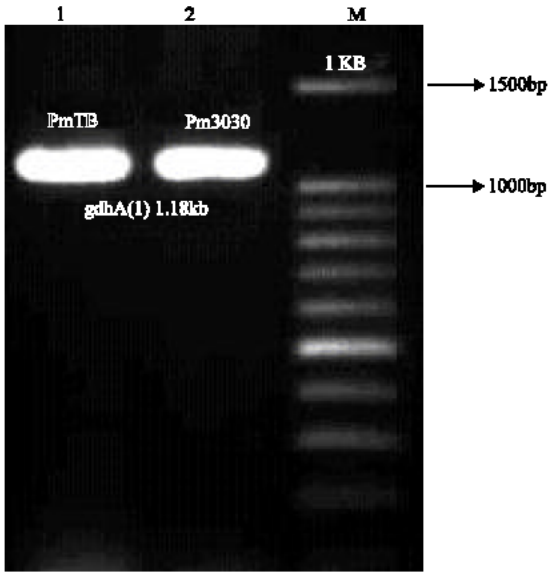

Fig. 2 The product following PCR with *gdh*A(1) primers showing the 1.8kb band of the whole *gdh*A genome. (M: marker 100bp DNA ladder (Promega, USA), Lane 1 and 2 were amplified PCR products from two isolates *P. multocida* B:2)

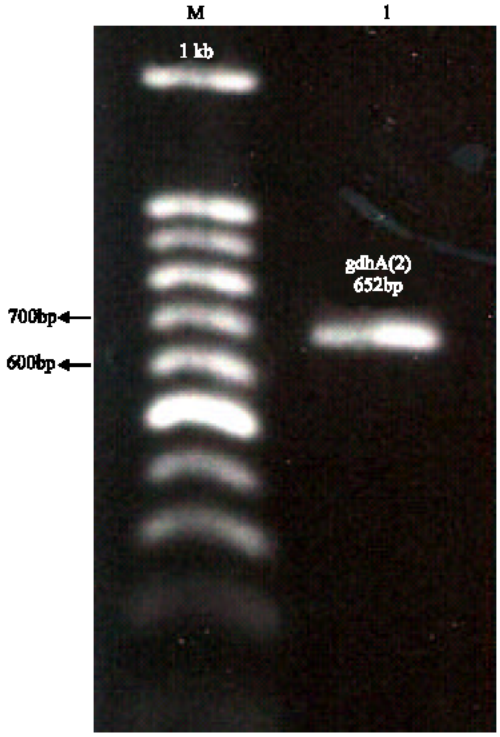

Fig. 3: The product following nested PCR with *gdhA* (2) primers showing the 652bp *gdh*A functional gene. (M: marker 100bp DNA ladder (Promega, USA), 1: Nested-PCR products of gdhA (2))

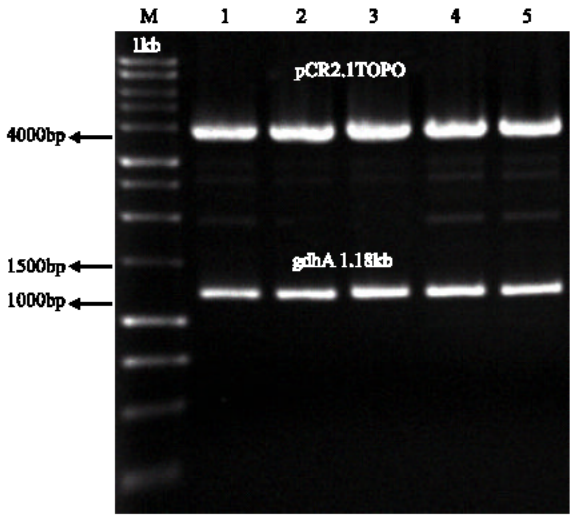

Fig. 4: Restriction enzyme digestion of pSZ1 using *Bam*HI and *Xho*I. The 3.9kb is the digested pCR2.1-TOPO vector and the 1.18kb is the digested *gdh*A gene. (M: GeneRuler™ 1kb DNA ladder (Fermentas, Lithuania), lane 1-5 were all positives clones)

DISCUSSION

The *gdhA* gene of bacteria has been associated with certain amino acid synthesis [9]. Therefore, manipulation of the gene will not disturb the viability of the bacteria. However, cells with disrupted *gdhA* gene, may become attenuated and glutamate dependant[9]. This characteristic may have a future use in the preparation of an attenuated live vaccine against haemorrhagic septicaemia of cattle and buffaloes[10].

Two sets of primers were designed in this study, based on the published *gdh*A sequence of *P. multocida* serotype A [4]. They were the *gdh*A [1] and *gdh*A [2] that successfully amplified the whole *gdh*A gene consisting of 1180bp and the functional part of the gene, which was 652bp. The products were then confirmed via sequencing.

Following sequencing, the PCR products were cloned with the TOPO TA Cloning Kit (Invitrogen, USA) resulting in pSZ1 plasmid. The plasmid was suitable to be used in this study because of the coordinated restriction enzyme sites of the insert. The suicide vector can later be used in the second phase cloning for the disruption of the *gdhA* gene of the host genome [11].

REFERENCES

1. Wijewardana, T.G., 1992. Haemorrhagic septicaemia. Rev. Med. Microbiol., 3: 59-63.
2. De Alwis, M.C.L., 1992. Haemorrhagic septicaemia-a general review. Br. Vet. J., 148: 99-112.
3. Tabatabaei, M., Z. Liu, A. Finucane, R. Parton and J. Coote, 2002. Protective immunity conferred by attenuated aroA derivatives of *Pasteurella multocida* B:2 strains in a mouse model of hemorrhagic septicemia. Infect. Immun., 70: 3355-3362.
4. Davies, R.L., R. MacCorquodale and S. Reilly, 2004. Characterisation of bovine strains of *Pasteurella multocida* and comparison with isolates of avian, ovine and porcine origin. Vet. Microbiol., 99: 145-58.
5. Helling, R.B., 1998. Pathway choice in glutamate synthesis in *Escherichia coli*. J. Bacteriol., 180: 4571-4575.
6. Belitsky, B.R., Hyun-Jin Kim and A.L. Soneshein, 2004. Ccp-A-dependant regulation of *Bacilus subtilis* glutamate dehydrogenase gene expression. J. Bacteriol., 186: 3392-3398.
7. Tartof, K.D. and C.A. Hobbs, 1987. Improved media for growing plasmid and cosmid clones. Bethesda Res. Lab. Focus, pp: 9-12.
8. Dower, W.J., J.F. Miller and C.W. Regsdale, 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res., 16: 6127-6145.
9. Hashim, S., D.H. Kwon, A. Abdelal and C.D. Lu, 2004. The arginine regulatory protein mediates repression by arginine of the operons encoding glutamate synthase and anabolic glutamate dehydrogenase in *Pseudomonas aeruginosa*. J. Bacteriol., 186: 3848-3854.
10. Verma, R. and T.N. Jaiswal, 1998. Haemorrhagic septicaemia vaccines. Vaccine, 16: 1184-1192.
11. Jablonski, L., N. Sriranganathan, S.M. Boyle and G.R. Carter, 1992. Conditions for transformation of *Pasteurella multocida* by electroporation. Microb. Pathog.,12: 63-68.